

# Genetic Differentiation in the Mitochondrial Cytochrome *b* Gene of Korean Brown Frog, *Rana dybowskii* (Amphibia: Ranidae)

Yu-Ri Kim, Dong-Eun Yang, Hyuk Lee, Jung-Eun Lee, Hyun-Ick Lee, Suh-Yung Yang, and Hei-Yung Lee\*

Department of Biology, College of Sciences, Inha University, Incheon 402-751, Korea

## Key Words:

Genetic differentiation  
Mitochondrial cytochrome  
*b* gene  
*Rana dybowskii*  
Sequence divergence

The nucleotide sequences of a 504 bp segment of the mitochondrial cytochrome *b* gene were analyzed to survey the intraspecific variation of the brown frog, *Rana dybowskii*, collected from nine populations in South Korea. Comparisons of sequence divergence of the cytochrome *b* gene suggest that the populations examined are clearly classified into two types (type 1 and type 2), diverged from each other by a high value of 14.3-15.9% sequence divergence. The two types are distributed allopatrically in most populations, but only one population occurs sympatrically. In the Tonghae population, their spawning grounds differ in that type 1 spawns in the puddle and type 2 spawns in the mountain creek. Based on the genetic divergences of the cytochrome *b* gene sequences, the phylogenetic status of Korean *R. dybowskii* is elucidated by comparing it with related brown frogs distributed in an area adjacent to the Korean Peninsula. Interspecific sequence divergences among type 1, type 2 and other related brown frog species (Russian *R. dybowskii*, *R. pirica*, *R. ornativentris*, *R. chensinensis*: 2n=24 chromosomes) used in this study ranged from 11.7 to 16.3%. *R. dybowskii* in Tsushima is very similar to our type 1 (sequence divergence=0-1.6%) and *R. chensinensis* in western China is closest to our type 2 (sequence divergence=6.8-7.5%).

Brown frogs consist of many species and they are morphologically very similar (Green and Borkin, 1993). Because of their phenotypic similarity, it is very difficult to classify some members with 24 chromosomes by the traditional method of morphological and karyological analyses. With the development of biochemical and molecular methods, phylogenetic relationships within and between species have been clarified (Green and Borkin, 1993; Tanaka et al., 1994, 1996; Sumida and Nishioka, 1996; Matsui et al., 1998; Tanaka-Ueno et al., 1998).

For Korean brown frogs with 24 chromosomes, Okada (1928) described three distinct species, *Rana temporaria coreana* n. subsp., *R. t. temporaria* and *R. t. ornativentris*. Kang and Yoon (1975) reexamined and identified them as *R. dybowskii* and *R. ornativentris*, but Yang and Yu (1978) reported that only *R. dybowskii* is distributed in South Korea and confirmed their contention in the study of osteology of Korean anurans (Yu and Yang, 1980).

In the present study, we intend to investigate the degree of inter- and intra-specific genetic variations

among populations of Korean *R. dybowskii* which have been a matter of debate by the previous authors, and to clarify the phylogenetic relationships between Korean *R. dybowskii* and other related brown frogs with 24 chromosomes.

## Materials and Methods

Collection sites and number of specimens from South Korea, China and Japan used in this study are listed in Fig. 1 and Table 1. Most specimens were collected from a single microhabitat such as a rice field, a puddle or a mountain creek. In Tonghae, however, the specimens were found in both puddle and mountain creek. These specimens from Tonghae were designated as two different groups (Tonghae A: puddle and Tonghae B: mountain creek) which were treated as separate operational taxonomic units (OTUs) in our analyses. For the comparison between brown frogs, published data of *R. ornativentris* and *R. pirica* from Japan, and *R. dybowskii* from Russia (Tanaka-Ueno et al., 1998) were used. *Xenopus laevis* (Dunon-Bluteau et al., 1985) and *R. catesbeiana* (Lee et al., unpublished data) were also included for outgroup comparison.

The total DNA was isolated from frozen (-70°C) tissue samples of liver, heart and intestine of each

\* To whom correspondence should be addressed.  
Tel: 82-32-860-7692, Fax: 82-32-874-6737  
E-mail: ihyung@inha.ac.kr

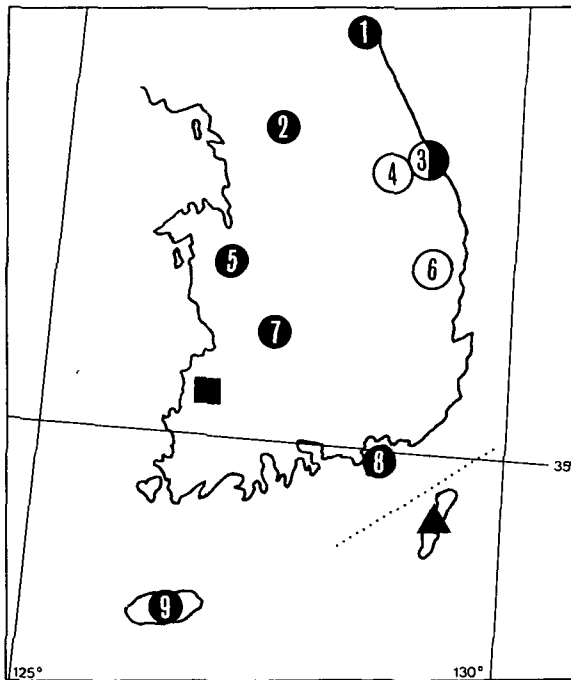


Fig. 1. Collection localities of samples for the mitochondrial cytochrome b gene of the type 1 (●) and type 2 (○) in Korea. Sympatric area (■), Jangsung (■, Oh, 1997) and Tsushima, Japan (▲). 1: Kosung, 2: Yangpyong, 3: Tonghae, 4: Jungsun, 5: Chungyang, 6: Chungsong, 7: Muju, 8: Koje, 9: Cheju.

specimen using extraction buffer [10 mM Tris-HCl, pH 8.0, 0.1 M EDTA, 20 µg/µl Pancreatic RNase, 0.5% SDS] (Sambrook et al., 1989).

A fragment of the mitochondrial cytochrome b gene (~560 base pairs) was amplified by PCR and the primers were as follows: CB1 (5'CCATCCAACATCTCA GCATGATGAAA3', modified from L14841 primer, Kocher et al., 1989); 560A (5'GTCTTTGTAGGAGAAGT ATGG3', H15410 primer, Tanaka et al., 1996). The amplified PCR product was sequenced directly using a TOP polymerase sequencing kit (Bioneer, Korea).

The DNA sequences were aligned by using DNASIS programs (Ver. 3.2, Hitachi Software Engineering Co. Ltd.). Basic statistics and genetic divergences among sequences based on the Tamura-Nei's distance (Tamura

and Nei, 1993) and Kimura's 2-parameter distance (Kimura, 1980) were estimated by using the MEGA program (Kumar et al., 1993). Using MEGA, UPGMA and neighbor-joining (NJ) phenogram with 1000 replications of bootstrapping test were obtained.

**Results**

Genetic differentiation of Korean and Japanese *R. dybowskii* and *R. chensinensis* from China were estimated using nucleotide sequences of the 504 bp region on the mitochondrial cytochrome b gene (Fig. 2). The sequences of *R. dybowskii* from Japan showed identical sequences with Yangpyong and Chungyang populations and the sequence similarity within two specimens of *R. chensinensis* from China was 98.4%.

Amino acid sequences were deduced from the nucleotide sequences of the 504 bp segment (Fig. 3). Although most nucleotide substitutions were silent mutations, four amino acids replacements occurred uniformly in Tonghae B, Chungsong and Jungson populations, and the 121th amino acid of the Kosung population was changed from isoleucine to methionine.

As shown in the dendrogram obtained by the UPGMA and neighbor-joining methods (Fig. 4A and B), the outgroup species of *R. catesbeiana* and *X. laevis* are clearly separated from ingroup species. The phenogram showed that there are two distinct genetic groups (type 1 and type 2) in Korean populations of *R. dybowskii*. The type 1 clustered with the Kosung, Tonghae A, Yangpyong, Chungyang, Muju, Koje, Cheju, and Japanese Tsushima populations, whereas type 2 clustered with the Tonghae B, Jungsun and Chungsong populations.

Sequence divergences of Tamura-Nei's distance and Kimura's 2-parameter distance were similar to each other. Each intrapopulation variation within type 1 and type 2 ranged from 0.0 to 0.0040 sequence divergence (Table 1). Interpopulation variation within type 1 and type 2 ranged from 0.0 to 0.0203 sequence divergence. However, sequence divergences between type 1 and type 2 ranged from 0.1425 to 0.1590.

For the phylogenetic analysis, our data and published data of *R. pirica*, *R. ornativentris* from Japan,

Table 1. Assayed species, collected localities and number of specimens

Species	Localities	No of specimens
<i>Rana dybowskii</i>	GoJin-up, Kosung-gun, Kangwon-do (Kosung)	3
	Ochun-ri, Yangpyong-gun, Kyonggi-do (Yangpyong)	3
	Ansung-myun, Muju-gun, Chollabuk-do (Muju)	3
	Jungsan-myun, Chungyang-gun, Chungchungnam-do (Chungyang)	3
	Chuwang mt., Chungsong-gun, Kyongsangbuk-do (Chungsong)	3
	Bichun-dong, Tonghae-shi, Kangwon-do (A) (Tonghae A)	3
	Bichun-dong, Tonghae-shi, Kangwon-do (B) (Tonghae B)	3
	Oban-dong, Jungsun-gun, Kangwon-do (Jungsun)	3
	Shinhyun-up, Koje-shi, Kyongsangnam-do (Koje)	3
	Cheju-shi, Cheju-do (Cheju)	3
<i>R. chensinensis</i>	Tokoya, Tsushima Isl., Ngasaki Prefecture, Japan (Tsushima 1)	1
	Shapotou, Yinnan prefecture, Ningxia Hui Autonomous Region, China (China 1)	1
	Lanzhou-Xining Rd., Hidong Prefecture, Qinghai Province, China (China 2)	1



Genetic Differentiation of Korean *Rana dybowskii*

	1		80
<i>Xenopus laevis</i>	QIIITGLFLAM HYTADTSMAF SSVAHICRDV NYGWLIRNLH ANGASFFFIC IYLHIGRGLY YGSFLYKETW NIGVILLFLV		
<i>Rana catesbeiana</i>	..V.....L...I.....N...L.....F.....Y.....		
<i>R. dybowskii</i> Muju 1/3	..A.....L...I...S.....N...L.....Y.....		
Muju 2	..A.....L...I...S.....N...L.....Y.....		
Koje 1/2/3	..A.....L...I...S.....N...L.....Y.....		
Kosung 1/2/3	..A.....L...I...S.....N...L.....Y.....		
Yangpyong 1/2/3	..A.....L...I...S.....N...L.....Y.....		
Chungyang 1/2/3	..A.....L...I...S.....N...L.....Y.....		
Cheju 1	..A.....L...I...S.....N...L.....Y.....		
Cheju 2	..A.....L...I...S.....N...L.....Y.....		
Cheju 3	..A.....L...I...S.....N...L.....Y.....		
Tonghae(A) 1/3	..A.....L...I...S.....N...L.....Y.....		
Tonghae(A) 2	..A.....L...I...S.....N...L.....Y.....		
Tsushima 1	..A.....L...I...S.....N...L.....Y.....		
Tonghae(B) 1/2/3	..A.....L...I.....N...L.....F.....Y.....		
Jungsun 1/2/3	..A.....L...I.....N...L.....F.....Y.....		
Chungsong 1/2/3	..A.....L...I.....N...L.....F.....Y.....		
<i>R. chensinensis</i> (China 1)	..A.....L...I.....N...L.....F.....Y.....		
<i>R. chensinensis</i> (China 2)	..A.....L...I.....N...L.....F.....Y.....		
<i>R. dybowskii</i> (Lazo, Russia)	..A.....Y.....N...L.....F.....Y.....		
<i>R. ornativentris</i> (Japan, mainland)	..A.....L...I.....N...L.....F.....Y.....		
<i>R. pirica</i> (Hokkaido, Japan)	..A.....L...I...S.....N...L.....F.....Y.....		
	81		168
<i>X. laevis</i>	NATAFVGYVL PWGQMSFWGA TVITNLLSAI PYIGNVLVQW IWGGFSVDNA TLTRFFAFHF LLPFIAGAS ILHLLFLHET GSTNPTGL		
<i>R. catesbeiana</i>	.....A...SD.....T...I.....A...MI.....Q...S.....		
<i>R. dybowskii</i> Muju 1/3	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Muju 2	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Koje 1/2/3	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Kosung 1/2/3	.....A...FD...M.....T...I.....AL...MI.....Q...S.....		
Yangpyong 1/2/3	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Chungyang 1/2/3	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Cheju 1	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Cheju 2	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Cheju 3	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Tonghae(A) 1/3	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Tonghae(A) 2	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Tsushima 1	.....A...FD.....T...I.....AL...MI.....Q...S.....		
Tonghae(B) 1/2/3	.....A...PD.....T...I.....AM...MI.....Q...S.....		
Jungsun 1/2/3	.....A...PD.....T...I.....AM...MI.....Q...S.....		
Chungsong 1/2/3	.....A...PD.....T...I.....AM...MI.....Q...S.....		
<i>R. chensinensis</i> (China 1)	.....A...SD.....T...I.....AM...MI.....Q...PS.....		
<i>R. chensinensis</i> (China 2)	.....A...SD.....T...I.....AM...MI.....Q...S.....		
<i>R. dybowskii</i> (Lazo, Russia)	.....A...FD.....T...I.....AV...MI.....Q...S.....		
<i>R. ornativentris</i> (Japan, mainland)	.....A...FD.....R.....T...I.....AM...MI.....Q...S.....		
<i>R. pirica</i> (Hokkaido, Japan)	.....A...FD.....R...H.....T...I.....AM...MI.....Q...S.....		

Fig. 3. Amino acid sequences encoded by 504 bp of the cytochrome *b* gene. Dots indicate identity to the sequence of *Xenopus laevis*.

and *R. dybowskii* from Russia were combined. In the UPGMA tree, these species from published data were clustered with our type 1 and type 2 grouped with *R. chensinensis*, but in the NJ tree, only *R. pirica* was clustered with type 1 and *R. ornativentris* was clustered with type 2 and *R. chensinensis*, and followed by the Russian *R. dybowskii*. Even though clustering patterns among them were different from each other, the bootstrap iteration values supporting their relationships ranged from 28% to 53%. The type 2 and *R. chensinensis* from China were grouped in 100% bootstrap support in the NJ and UPGMA tree and sequence divergences between them ranged from 0.0681 to 0.0748.

Discussion

Although Korean 2n=24 brown frog populations resembled one another morphologically, mitochondrial haplotypes were separated into two groups (type 1 and type 2) and the mean sequence divergence between two types was 14.8%. Interspecific sequence divergences among type 1, type 2 and other related brown frog species (Russian *R. dybowskii*, *R. pirica*, *R. ornativentris*, *R. chensinensis*; 2n=24 chromosomes) used in this

study ranged from 11.7 to 16.3%. Oh (1997) provided 357 bp sequences of the cytochrome *b* gene for *R. dybowskii* from Jangsung. The sequences at the 61-357th positions corresponded to the 1-297th positions of our type 2 and differed only by 8 bp (sequence similarity=97.3%). In isozyme analysis, Jangsung population was separated with two types of genetic groups (Yang et al., unpublished data). The results of our study and genetic divergences among congeneric species of amphibia (Garcia-Paris et al., 1998; Johns and Avise, 1998) suggest that two types of Korean *R. dybowskii* are separate species which are distinctively different from related brown frogs.

In the both UPGMA and NJ trees, these relationships were supported strongly by 100% of bootstrap iterations (Fig. 4A, B). In the California Newt, *Taricha torosa*, sequence divergences among subspecies ranged from 7.0 to 9.0% (Tan and Wake, 1995). A similar degree of genetic divergence was recognized between *Rana nigromaculata* and *Rana plancyi* (sequence divergence=4.0-6.8%) (Lee et al., 1997). *R. nigromaculata* and *R. plancyi* are distinct sympatric species, although *R. plancyi* was once considered as a subspecies of *R. nigromaculata* (Okada, 1928).

Type 2 was clustered with *R. chensinensis* from

**Table 2.** Pairwise matrix of nucleotide sequence divergence estimated by Tamura-Nei distance (below diagonal) and Kimura's two-parameter distance (above diagonal)

	1	2	3	4	5	6	7	8	9	10	11
1. <i>Xenopus laevis</i>		0.2573	0.2937	0.2937	0.2969	0.2934	0.2937	0.2937	0.3001	0.3033	0.3033
2. <i>Rana catesbeiana</i>	0.2631		0.1908	0.1908	0.1936	0.1905	0.1908	0.1908	0.1908	0.1936	0.1963
3. Muju 1/3	0.3038	0.1958		0.0020	0.0040	0.0040	0.0020	0.0020	0.0141	0.0161	0.0182
4. Muju 2	0.3038	0.1958	0.0020		0.0060	0.0060	0.0040	0.0040	0.0161	0.0182	0.0202
5. Kojje 1/2/3	0.3076	0.1990	0.0040	0.0060		0.0040	0.0020	0.0020	0.0100	0.0120	0.0141
6. Kosung 1/2/3	0.3029	0.1952	0.0040	0.0060	0.0040		0.0020	0.0020	0.0141	0.0161	0.0181
7. Yangpyong 1/2/3	0.3037	0.1958	0.0020	0.0040	0.0020	0.0020		0.0000	0.0120	0.0141	0.0161
8. Chungyang 1/2/3	0.3037	0.1958	0.0020	0.0040	0.0020	0.0020	0.0000		0.0120	0.0141	0.0161
9. Cheju 1	0.3103	0.1966	0.0141	0.0161	0.0100	0.0141	0.0121	0.0121		0.0020	0.0040
10. Cheju 2	0.3129	0.1990	0.0161	0.0182	0.0121	0.0141	0.0141	0.0141	0.0020		0.0020
11. Cheju 3	0.3130	0.2022	0.0182	0.0203	0.0141	0.0182	0.0161	0.0161	0.0040	0.0020	
12. Tonghae(A) 1/3	0.3038	0.1990	0.0040	0.0060	0.0040	0.0040	0.0020	0.0020	0.0141	0.0161	0.0141
13. Tonghae(A) 2	0.3000	0.2022	0.0060	0.0080	0.0060	0.0060	0.0040	0.0040	0.0161	0.0182	0.0161
14. Tsushima 1	0.3037	0.1958	0.0020	0.0040	0.0020	0.0020	0.0000	0.0000	0.0121	0.0141	0.0161
15. Tonghae(B) 1/2/3	0.3071	0.2110	0.1561	0.1561	0.1504	0.1556	0.1533	0.1533	0.1425	0.1449	0.1476
16. Jungsun 1/2/3	0.3071	0.2110	0.1561	0.1561	0.1504	0.1556	0.1533	0.1533	0.1425	0.1449	0.1476
17. Chungsong 1/2/3	0.3071	0.2110	0.1561	0.1561	0.1504	0.1556	0.1533	0.1533	0.1425	0.1449	0.1476
18. <i>R. chensinensis</i> (China 1)	0.3091	0.2045	0.1561	0.1561	0.1505	0.1528	0.1533	0.1533	0.1538	0.1561	0.1533
19. <i>R. chensinensis</i> (China 2)	0.3146	0.2174	0.1504	0.1504	0.1449	0.1472	0.1476	0.1476	0.1528	0.1552	0.1524
20. <i>R. dybowskii</i> (Lazo, Russia)	0.3147	0.1855	0.1463	0.1463	0.1407	0.1430	0.1435	0.1435	0.1376	0.1400	0.1373
21. <i>R. ornativentris</i> (Japan, mainland)	0.3536	0.1915	0.1370	0.1370	0.1343	0.1394	0.1370	0.1370	0.1320	0.1343	0.1317
22. <i>R. pirica</i> (Hokkaido, Japan)	0.3503	0.2138	0.1196	0.1196	0.1196	0.1193	0.1170	0.1170	0.1173	0.1196	0.1222

	12	13	14	15	16	17	18	19	20	21	22
1. <i>X. laevis</i>	0.2937	0.2906	0.2937	0.2987	0.2987	0.2987	0.2991	0.3060	0.3044	0.3312	0.3406
2. <i>R. catesbeiana</i>	0.1936	0.1963	0.1908	0.2061	0.2061	0.2061	0.1980	0.2118	0.1806	0.1850	0.2095
3. Muju 1/3	0.0040	0.0060	0.0020	0.1537	0.1537	0.1537	0.1540	0.1484	0.1443	0.1355	0.1189
4. Muju 2	0.0060	0.0080	0.0040	0.1537	0.1537	0.1537	0.1540	0.1484	0.1443	0.1355	0.1189
5. Kojje 1/2/3	0.0040	0.0060	0.0020	0.1484	0.1484	0.1484	0.1487	0.1432	0.1391	0.1330	0.1189
6. Kosung 1/2/3	0.0040	0.0060	0.0020	0.1534	0.1534	0.1534	0.1510	0.1456	0.1414	0.1379	0.1186
7. Yangpyong 1/2/3	0.0020	0.0040	0.0000	0.1510	0.1510	0.1510	0.1510	0.1458	0.1417	0.1355	0.1164
8. Chungyang 1/2/3	0.0020	0.0040	0.0000	0.1510	0.1510	0.1510	0.1513	0.1458	0.1417	0.1355	0.1164
9. Cheju 1	0.0141	0.0161	0.0120	0.1407	0.1407	0.1407	0.1513	0.1510	0.1365	0.1305	0.1164
10. Cheju 2	0.0161	0.0182	0.0141	0.1432	0.1432	0.1432	0.1540	0.1537	0.1391	0.1330	0.1189
11. Cheju 3	0.0141	0.0161	0.0161	0.1458	0.1458	0.1458	0.1513	0.1510	0.1365	0.1305	0.1213
12. Tonghae(A) 1/3	0.0020	0.0020	0.0020	0.1537	0.1537	0.1537	0.1487	0.1432	0.1391	0.1330	0.1189
13. Tonghae(A) 2	0.0020	0.0040	0.0040	0.1563	0.1563	0.1563	0.1513	0.1458	0.1417	0.1355	0.1213
14. Tsushima 1	0.0020	0.0040	0.0040	0.1510	0.1510	0.1510	0.1513	0.1458	0.1417	0.1355	0.1164
15. Tonghae(B) 1/2/3	0.1561	0.1590	0.1533	0.0000	0.0000	0.0000	0.0675	0.0742	0.1593	0.1435	0.1534
16. Jungsun 1/2/3	0.1561	0.1590	0.1533	0.0000	0.0000	0.0000	0.0675	0.0742	0.1593	0.1435	0.1534
17. Chungsong 1/2/3	0.1561	0.1590	0.1533	0.0000	0.0000	0.0000	0.0675	0.0742	0.1593	0.1435	0.1534
18. <i>R. chensinensis</i> (China 1)	0.1505	0.1533	0.1533	0.0681	0.0681	0.0681	0.0203	0.0202	0.1412	0.1264	0.1407
19. <i>R. chensinensis</i> (China 2)	0.1449	0.1476	0.1476	0.0748	0.0748	0.0748	0.0203	0.0202	0.1461	0.1261	0.1409
20. <i>R. dybowskii</i> (Lazo, Russia)	0.1407	0.1435	0.1435	0.1603	0.1603	0.1603	0.1426	0.1474		0.1358	0.1312
21. <i>R. ornativentris</i> (Japan, mainland)	0.1343	0.1370	0.1370	0.1454	0.1454	0.1454	0.1276	0.1270	0.1363		0.1257
22. <i>R. pirica</i> (Hokkaido, Japan)	0.1196	0.1222	0.1170	0.1552	0.1552	0.1552	0.1418	0.1419	0.1315	0.1260	

3-17; *Rana dybowskii* populations.

western China (sequence divergence=6.8-7.5%). *R. chensinensis* was originally described from Tsingling, Shaanxi, China and distribution of this species ranges from northern China towards the south to Jiangu in the east and Sichuan in the west, as well as in Russia, Korea and eastern Mongolia (Zhao and Adler, 1993). Shannon (1956) applied the name *dybowskii* to brown frogs in the northeast area of China against the distribution of *R. chensinensis*, so that he described the Korean brown frogs as *R. temporaria dybowskii*. The distribution of *R. dybowskii* is Abrek Bay, Maritime Territory, but neither our type 1 nor type 2 correspond to this species.

Okada (1928) referred to brown frogs with 24 chromosomes distributed in North Korea as *R. pirica*. In contrast, Matsui et al. (1998) presumed that these North Korean brown frogs may be identical to *R. dybowskii* from the Maritime Territory by geographic distribution and molecular evidence. Although previous opinions are in conflict about the presence and

taxonomic status of brown frogs in North Korea, the existence of the two types of brown frogs in South Korea have not been reported. If our type 2 is the northern form as they described, their suggestions did not match with our results.

Although morphological and ecological characters are generally very similar among brown frogs, microhabitats and spawning sites are utilized for the classification of Japanese brown frogs (Maeda and Matsui, 1989). Distinct differentiation of spawning site and shape of egg sac were observed between Tonghae populations of type 1 and type 2. Also, these two types differ in their ventral color pattern (Yang et al., unpublished data). These facts support our further efforts to seek for more ecological and morphological characters to identify these two types and to infer their phylogenetic status. Also more detailed studies of *R. chensinensis* and related subspecies which are distributed in areas adjacent to the Korean Peninsula, especially North Korea, will be needed for phylogenetic

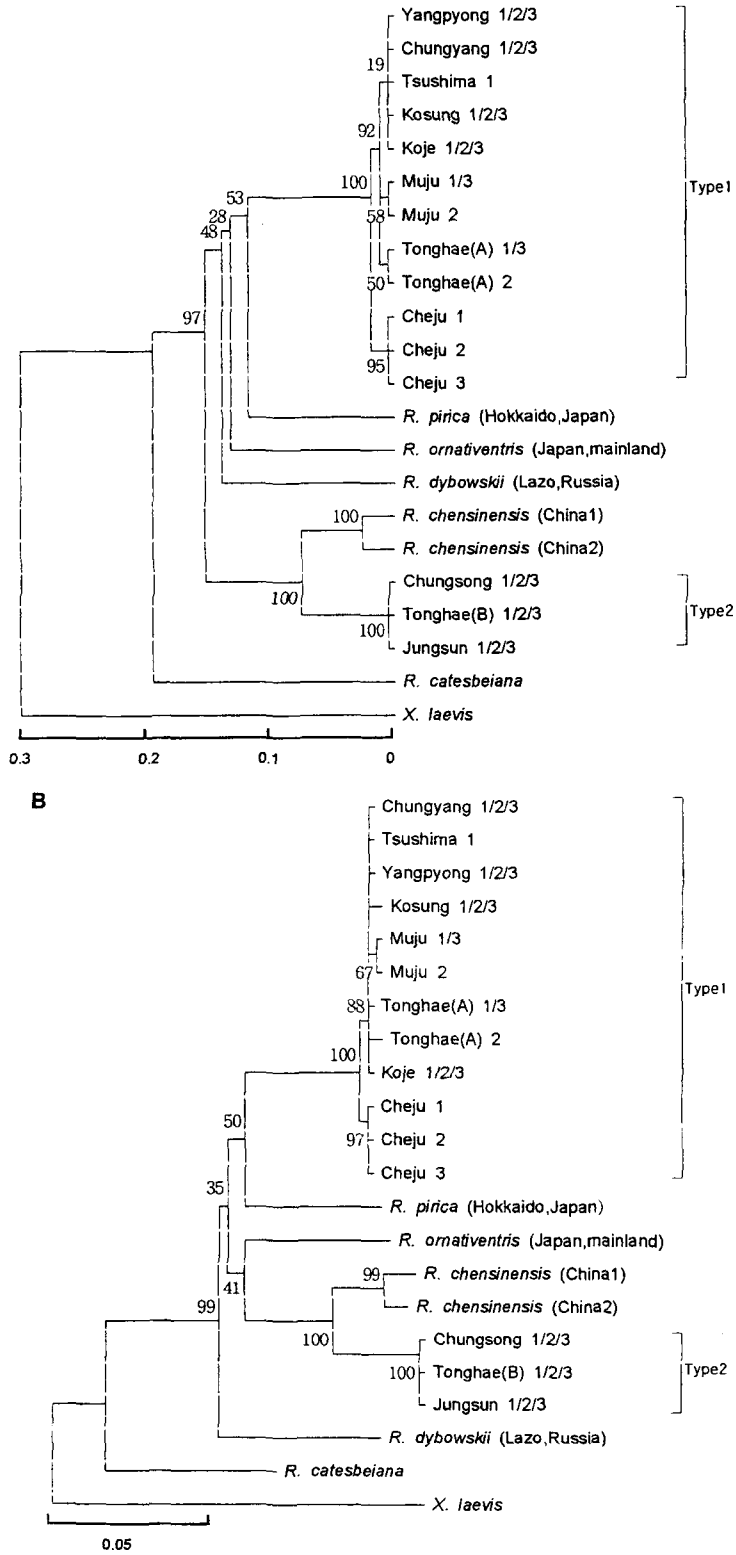


Fig. 4. A UPGMA (A) and a neighbor-joining (B) phenogram of Korean *Rana dybowskii* (at 9 localities), a Japanese *R. dybowskii* and related species from adjacent regions. Branch lengths represent the sequence divergence estimated by Tamura-Nei's algorithm and the nodal values indicate percent supports in 1000 bootstrap replications.

relationship analysis.

Combining variable data set is useful in reconstructing phylogenetic relationships among closely related species (Zink and Blackwell, 1996; Montgelard et al., 1997; Todd et al., 1997; Bloomer and Crowe, 1998). Analyses by combining isozyme, morphological, ecological and nucleotide sequence data will provide stronger evidence for the phylogenetic hypothesis and classification, especially for our type 2 of the Korean *Rana dybowskii*.

#### Acknowledgements

This research was supported by a grant from the Korean Ministry of Education of Korea (BSRI-97-4423).

#### References

- Bloomer P and Crowe TM (1998) Francolin phylogenetics: molecular, morphobehavioral, and combined evidence. *Mol Phylogenet Evol* 9: 236-254.
- Dunon-Bluteau D, Volovitch M, and Brun G (1985) Nucleotide sequence of a *Xenopus laevis* mitochondrial DNA fragment containing the D-loop, flanking tRNA genes and apocytochrome *b* gene. *Gene* 36: 65-78.
- Garcia-Paris M, Alcobendas M, and Alberch P (1998) Influence of the Guadalquivir river basin on mitochondrial DNA evolution of *Salamandra salamandra* (Caudata: Salamandridae) from southern Spain. *Copeia* 1998: 173-176.
- Green DM and Borkin LJ (1993) Evolutionary relationships of eastern Palearctic brown frogs, genus *Rana*: paraphyly of the 24-chromosome species group and the significance of chromosome number change. *Zool J Linn Soc* 109: 1-25.
- Johns GC and Avise JC (1998) A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. *Mol Biol Evol* 15: 1481-1490.
- Kang YS and Yoon IB (1975) Illustrated Encyclopedia of Fauna and Flora of Korea. Vol 17. Amphibia and Reptilia. Ministry of Education of Korea, Seoul. pp 98-103.
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111-120.
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, and Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: application and sequencing with conserved primers. *Proc Natl Acad Sci USA* 86: 6196-6200.
- Kumar S, Tamura K, and Nei M (1993) MEGA: Molecular Evolutionary Genetics Analysis, Version 1.01. Pennsylvania State University, Pennsylvania.
- Lee HY, Oh SJ, Yang DE, Kim YR, and Yang SY (1997) Genetic differentiation between two species in the genus *Rana* of Korea. *Korean J Genet* 19: 317-324.
- Maeda N and Matsui M (1989) Frogs and Toads of Japan. Bun-ichi Sogo Shuppan, Tokyo, pp 44-83.
- Matsui M, Tanaka-Ueno T, Paik NK, Yang SY, and Takenaka O (1998) Phylogenetic relationships among local populations of *Rana dybowskii* assessed by mitochondrial cytochrome *b* gene sequences. *Jpn J Herpetol* 17: 145-151.
- Montgelard C, Catzeffis FM, and Douzery E (1997) Phylogenetic relationships of artiodactyls and cetaceans as deduced from the comparison of cytochrome *b* and 12S rRNA mitochondrial sequences. *Mol Biol Evol* 14: 550-559.
- Oh SJ (1997) The analysis of partial sequences of cytochrome *b* gene in four species of Korean *Rana*. MS Thesis, Inha University, Incheon. pp 1-48.
- Okada Y (1928) Frogs in Korea. *J Chosen Nat Hist Soc* 6: 15-46.
- Sambrook J, Fritsch EF, and Maniatis T (1989) Molecular Cloning: a Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Shannon FA (1956) The reptiles and amphibians of Korea. *Herpetologica* 12: 22-49.
- Sumida M and Nishioka M (1996) Genetic variation and population divergence in the mountain brown frog *Rana ornativentris*. *Zool Sci* 13: 537-549.
- Tamura K and Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in human and chimpanzees. *Mol Biol Evol* 10: 512-526.
- Tan AM and Wake DB (1995) Mt DNA phylogeography of the California Newt, *Taricha torosa* (Caudata, Salamandridae). *Mol Phylogenet Evol* 4: 383-394.
- Tanaka T, Matsui M, and Takenaka O (1994) Estimation of phylogenetic relationships among Japanese brown frogs (Amphibia: Anura) from mitochondrial cytochrome *b* gene. *Zool Sci* 11: 753-757.
- Tanaka T, Matsui M, and Takenaka O (1996) Phylogenetic relationships of Japanese brown frogs (*Rana*: Ranidae) assessed by mitochondrial cytochrome *b* gene sequences. *Biochem Syst Ecol* 24: 299-307.
- Tanaka-Ueno T, Matsui M, Sato T, Takenaka S, and Takenaka O (1998) Phylogenetic relationships of brown frogs (*Rana*: Ranidae) with 24 chromosomes from far east Russia and Hokkaido assessed by mitochondrial cytochrome *b* gene sequences. *Zool Sci* 15: 289-294.
- Todd RJ, Applebaum G, and Wake DB (1997) Phylogenetic relationships of bolitoglossine salamanders: a demonstration of the effects of combining morphological and molecular data sets. *Mol Biol Evol* 14: 883-891.
- Yang SY and Yu CH (1978) Check list of Korean amphibians. *Inst Ind Res Inha Univ, Incheon* 5: 81-90.
- Yu CH and Yang SY (1980) Osteology of Korean anurans. *Basic Sci Res Inst Inha Univ, Incheon* 1: 81-87.
- Zhao EM and Adler K (1993) Herpetology of China. Oxford Press, Ohio, p 95, pp 140-141.
- Zink RM and Blackwell RC (1996) Patterns of allozyme, mitochondrial DNA, and morphometric variation in four sparrow genera. *Auk* 113: 59-67.

[Received March 4, 1999; accepted April 16, 1999]